

soil. The phase microscope is of little help with unstained preparations at low magnifications. With stained preparations the colour changes due to the phase arrangement suppress the contrast between green and red. Therefore it is not useful for counting purposes.

The recovery tests showed that with pure cultures a good recovery was obtained (Table 1). When a mixture of several pure cultures was added to a soil the recovery was reasonable (Table 2).

When the present technique was compared with culturing techniques (Table 3), it was found that a great number of protozoa which were missing in culture were estimated by the direct method. Also culture techniques involving pre-treatment of soil with acid do not provide a reliable estimate of the resting forms of protozoa which can be observed and counted by direct microscopy.*

The lowest theoretical number of protozoa which can be estimated in soil by this technique is 10 cells per gram. It is unlikely that such a small population would be of any significance. When the number reaches 100 or more there is no difficulty in obtaining a reliable count. Usually satisfactory agreement is obtained between duplicates.

CONCLUSIONS.

The technique described is a suitable method for the estimation of protozoan populations in soils. It has the advantage of providing a non-selective estimate of the groups making up the total population in any soil. The staining method is not limited to studies of protozoa. It may be used also for examining bacteria and fungi. When used in conjunction with the Gram stain it enables more detailed information to be collected on the soil microflora. For field experiments the slides can be prepared in the field and studied later in the laboratory without allowing any change in the protozoan population during transport.

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* Unfortunately it is not possible to differentiate the cysts of different protozoa, nor to identify with accuracy the different protozoa by the direct microscopy.

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EXPLANATION OF PLATE VI, B.

- (a) Ciliate: Cilia are readily visible on the original print.
- (b) Flagellate: The print is over-exposed to show the flagellum. Otherwise some internal structure would be visible.
- (c) Amoebae.

(Photographs taken at an initial magnification of 270× and enlarged to 950× for reproduction.)

SYSTEMATIC STATUS OF A LEAF RUST ON *HORDEUM LEPORINUM* LINK IN AUSTRALIA.

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[Read 29th June, 1955.]

Synopsis.

A rust species, *Puccinia hordei* Fckl., occurring on *Hordeum leporinum* Link in the Hills district near Sydney, has been identified by morphological and pathological studies.

The nomenclature of this rust as *P. hordei* Fckl. conflicts with the current nomenclature of the dwarf leaf rust of barley, now known as *P. hordei* Otth.; the epithet "*hordei*" for the rust species on *H. leporinum* has priority. Buchwald's suggestion that this rust be relegated to subspecific status with the name *P. hordei-murini* is discussed. It is concluded that there is no evidence to support this suggestion and that the present confusion can be dispelled only by experimental studies with the alternate host plants.

INTRODUCTION.

Hordeum leporinum Link has frequently been reported as a host for various species of rust in Australia and other parts of the world. These have included the species *Puccinia graminis*, subspecies *avenae* E. & H. and *tritici* E. & H. (Waterhouse, 1952), *P. glumarum* (Schm.) E. & H. and *P. hordei* Fckl. (not *P. hordei* Otth.) (Straib, 1937; Arthur, 1934). In glasshouse tests Waterhouse (1929) listed the species as susceptible to *P. simplex* E. & H. (syn. *P. hordei* Otth.) and *P. trititina* Erikss. This was in contrast to an earlier report (Waterhouse, 1927) in which *H. murinum* L. was highly resistant to *P. anomala* Rostr. (syn. *P. hordei* Otth.). Other authors in Australia have reported *H. murinum* to be uniformly resistant to *P. hordei* Otth., the dwarf leaf rust of barley (Watson and Butler, 1948).¹

A specific leaf rust on *H. murinum* (now almost certainly taxonomically *H. leporinum*) was first described by Fuckel in 1860 (Arthur, 1934; Cunningham, 1931). Straib (1937), on the basis of inoculation tests, showed this rust to be distinct from *P. dispersa* E. & H., *P. trititina* and *P. simplex* (syn. *P. hordei* Otth.). Buchwald (1943) and Arthur (1934) have emphasized that the rust *P. hordei* Fckl., is morphologically distinct from the rust *P. hordei* Otth., on cultivated barley.

OCCURRENCE.

A specimen of *H. leporinum* bearing leaf rust was collected at Castle Hill Research Station in August, 1954. The systematic status of the plant was verified by the junior author. The plant had 14 pairs of chromosomes and is thus differentiated from the closely related *H. murinum* L. and *H. stebbinsii* Covas, each of which has seven pairs of chromosomes (Covas, 1949).

H. leporinum was not widespread at Castle Hill, but most plants were lightly infected with rust herein styled Acc. No. 54.40. The dark orange uredosori were mostly epiphyllous, occasionally on both leaf surfaces, scattered over the leaf blade, raised and opening by a narrow longitudinal slit in the epidermis. A mild epiphytotic of the disease was later observed in a dense stand of *H. leporinum* at a neighbouring centre, Baulkham Hills. In this material uredosori frequently occurred on the leaf sheaths, as well as laminae, and plants were severely damaged.

¹ The specific designation *H. murinum* L. was presumably used by Waterhouse (1927 and 1929) for *H. leporinum* Link on Covas' scheme (Covas, 1949) for the classification of the genus *Hordeum*, whilst Watson and Butler (1948) use the two specific names synonymously.

Inoculation tests with the rust collected at Castle Hill and morphological studies of the uredospores indicate that the rust closely resembles the form described by Fuckel (Arthur, 1934). A critical identification based on the teleutospores has not been possible, since these have not yet been observed.

SPECIALIZATION AND HOST RANGE.

Seedlings and/or mature plant clones of the following grass species were inoculated with fresh uredospores taken from pustules developing on *H. leporinum* seedlings in the glasshouse; in no instance was there any indication of infection (infection type "O"):

Aegilops divaricata,¹ *Agrostis avenacea* Gmel., *A. tenuis* Sibth., *A. tenuis* var. *aristata* (Parnell) Druce, *Bromus mollis* L., *B. hordeaceus* L., *Cynodon dactylon* (L.) Pers. (seedlings and mature plants), *Danthonia semiannularis* (Labill.) R. Br., *Festuca rubra* L. var. *fallax* Hack., *Holcus lanatus* L. (mature plant), *Lolium perenne* L., *L. multiflorum* Lam., *L. rigidum* Gaud., *Lolium* sp. (N.Z. short rotation), *Poa pratensis* L., *Stipa aristiglumis* F. Muell., *S. verticillata* Nees.

No infection was observed on inoculated seedlings of Federation wheat, Black Winter rye, Algerian oats or Burke oats (infection type "O").

Three collections of *H. spontaneum* Koch. in the seedling stage gave a necrotic fleck reaction (infection type ";;").

Seedlings of one collection of *H. bulbosum* L. and two of *H. maritimum* Huds. gave a few necrotic flecks (infection type "O;;").

The results of the remaining inoculation tests with species of *Hordeum* are given in Table 1. Approximately 30 seedlings from bulked progenies of each variety were tested in each case, except for *H. leporinum* collections from Newtown and Binnaway, N.S.W., where one single adult plant was tested in each instance and also where seedlings of single plant progenies of two collections of this species made in the University grounds were used. The tests were conducted during the early summer months in the glasshouse within a temperature range of 60°–80°F. approx.

Inoculation results indicate that the rust on *H. leporinum* does not conform to any of the more commonly occurring rusts in Australia. An occasional infection with a low reaction type was observed on cultivated barley, but the differentiation from the known races of *P. hordei* Otth. is evident from the low infection types on all varieties tested. Moreover, in reciprocal tests, *H. leporinum* showed only a few necrotic flecks when inoculated with UN (unified numeration) 16 and UN 14, the two races of *P. hordei* Otth. present in Australia, following the differential set of varieties and key used by Levine and Cherevick (1952).

The inoculation results on wheat, oats, and rye differentiate the rust from *P. tritici*, *P. coronata avenae* Erikss. and *P. dispersa*.

MORPHOLOGY.

In morphological studies accession number 5440 was compared with race UN16, the common Australian race of *P. hordei* Otth. Uredospore measurements were made for 100 spores shaken on a slide from fully developed cultures of each rust and mounted in 50 per cent lactic acid. The results are given in Table 2.

Uredospores of the rust on *H. leporinum* are significantly smaller and lighter in colour than those of race UN16 of *P. hordei* Otth.

Considering the rust identified on *H. leporinum* elsewhere, the uredospore measurements closely correspond to those given for *P. hordei* Fckl. by Arthur (1934), viz., "18–24 by 22–28 μ ". The ranges recorded for the present isolate were 18.8–23.2 by 21.8–29.0 μ .

The shape of the uredospore was ellipsoid or slightly subovoid and the germ tubes were indistinct with probably three or more in a face.

The uredospore size is probably significantly different from that recorded for *P. glumarum* by Arthur (1934). The distribution of uredosori on the leaf may also

¹ Initially introduced from Cambridge, England, as *A. divaricata*, subsequently determined by Dr. A. E. Watkins to be *Triticum dicoccum* Schulb.

serve to distinguish *P. glumarum* from accession No. 50.40 on *H. leporinum*, as also do inoculation results with wheat, oats, rye, barley and *Bromus* species. In addition, the uredospore size and shape, coupled with inoculation results, indicate that the rust is probably distinct from any of the remaining rust species on graminaceous plants described by McAlpine (1906).

TABLE 1.
Results of Seedling Inoculation of Species of Hordeum with Rust Accession S.U. 54.40.

Species and Variety.	Infection Type.	Class of Reaction.
<i>Hordeum leporinum</i> —1 ¹	4	Susceptible.
" " —2 ¹	4	"
" " 3	4	"
" " 3	4	"
<i>H. vulgare</i> L. emend. Lam. ⁴		
Smooth awn × Manchuria B36 ⁵	0	Immune.
No. 22 B69	0	"
No. 49 B62	0	"
Athos B125	0	"
Cape B46	; 1 ⁻	Very resistant.
<i>H. distichum</i> L. emend. Lam.		
Kinver B49	0	Immune.
Goldfoil B167	0	"
Purple nudum C.I.2250 B28	; 1 ⁻ 1 ⁻	Very resistant.
<i>H. irregulare</i> E. Åberg and Wiebe		
Abate—Red stem B308	; 1 ⁻ 1	Resistant.
Abyssinian intermediate B490	; 1 ⁻	Very resistant.

¹ Progeny of single plant selection, Sydney University.

² Adult plant, collected Newtown, Sydney.

³ Adult plant, collected Binnaway, N.S.W.

⁴ Classifications follow the system of E. Åberg and G. Wiebe (1946).

⁵ Sydney University accession number.

On the basis of these results it seems reasonable to conclude, even in the absence of a study of the teleutospores, that the present rust on *H. leporinum* belongs to the species tentatively termed *P. hordei* Fckl.

TABLE 2.
Comparison of Studies with Two Rust Cultures.

Character.	<i>P. hordei</i> Otth. Race UN16 (Culture S.U. No. 62) from Barley.	Rust Accession 54.40 from <i>H. leporinum</i> .
Uredosori colour	Dragon's-blood Red ¹ — Plate XIII.	Rufous—Plate XIV.
Uredospore colour (low power, full illumination)	Ochraceous-Orange— Plate XV.	Antimony Yellow— Plate XV.
Uredospore length ..	26.7 ± 0.16 μ ²	24.7 ± 0.15 μ
Uredospore width ..	23.9 ± 0.14 μ ²	21.4 ± 0.1 μ
Cytoplasm of uredospore ..	Aggregated along spore wall.	Aggregated in centre of spore.

¹ Ridgway's Color Standards (1912).

² Significantly different at P = 0.05.

SYSTEMATICS AND DISCUSSION.

So far as the authors are aware, there has been no previous record of the occurrence of *P. hordei* Fckl. on *H. leporinum* in this country. However, Professor W. L. Waterhouse recalls (personal communication) the examination of rust on *H. leporinum* which was

unable to produce infection on wheat, oats, rye or barley. This probably was the same species as that considered here, and it is not unlikely that the rust has been present in Australia for some time.

The systematic status of this rust, tentatively termed *Puccinia hordei* Fckl., has now to be considered. The use of the epithet "*hordei*" conflicts with the correct name of *P. hordei* Otth. for the dwarf leaf rust of barley. Use of the latter name dates from 1952, when Levine and Cherewick (1952) reviewed the nomenclature of this pathogen. Briefly, this can be summarized as follows.

Winter in 1884 classified the leaf rust on barley as *P. rubigo-vera* var. *simplex* Körnicke. Eriksson and Henning raised it to specific rank, with the name *P. simplex*, in 1894. However, this name was applied by Peck in 1881 to a different species and was moreover antedated by the name *P. anomala* published by Rostrup in 1878 (Cunningham, 1931). The name *P. hordei* Otth. was published in 1871, and Stevenson and Johnson (cited by Levine and Cherewick (1952)) give this name priority. Yet Cunningham (1931) states that this name could not be used since it was applied by Fückel in 1860 to a "different and valid species".

Buchwald (1943) resolved this conflict by relegating the rust on *H. murinum* described by Fückel to subspecific status with the name *P. hordei-murini*. This would require that the rusts on cultivated barley and wild barley grasses should have a common alternate host plant. Buchwald based this contention on the apparently faulty evidence that "*P. hordei* Fckl. 1873 is a homonym of *P. hordei* Otth. 1871". The *P. hordei* of Fückel was described in 1860 (Fückel, 1860, cited by Arthur, 1934). No reference to the aecidial stage of either rust was made.

Obviously the conflicting nomenclature of these rusts can be corrected only by experimental studies with the alternate host plants. The aecidial stage of *P. hordei* Fckl. is not yet known (Arthur, 1934). The aecidial stage of *P. hordei* Otth. has been produced on *Ornithogalum umbellatum* L., *O. narbonense* L., *O. pyrenaicum* L., and on *Dipcadi serotinum* (L.) Medic. (d'Oliveira, 1939; Mains and Jackson, 1924; Mains, 1930; Dennis and Sandwith, 1948; d'Oliveira, 1949; and Levine and Cherewick, 1952) in Europe and North America. In Australia the teleutospores could not be germinated and the natural occurrence of the aecidial stage has not been observed (Waterhouse, 1952). This will need to be done if the nomenclature of these rusts is to be lifted above the present level of confusion.

It is concluded from the above review that the name "*P. hordei*" for the rust on *H. leporinum* has priority and the ultimate retention of *P. hordei* Otth. for the dwarf leaf rust of barley will require the verification of *P. hordei-murini* (Fckl.) Buch. as a valid subspecies.

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